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Fetal and adult human oligodendrocyte progenitor cells effectively myelinate dysmyelinated brain

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Both the late gestation and adult human forebrain contain large numbers of oligodendrocyte progenitor cells (OPCs). These cells may be identified by the phenotype A2B5$^+$/PSA-NCAM$^-$. We used dual color FACS to extract OPCs from 21-23 week fetal human forebrain, and A2B5 selection alone to extract these cells from adult white matter. Upon xenograft to the forebrains of newborn shiverer mice, the fetal OPCs dispersed throughout the white matter, and developed as oligodendrocytes and astrocytes. By 12 weeks, the host brains manifested extensive myelin production, compaction, and axonal myelination. Isolates of OPCs derived from the adult human white matter were also found to myelinate shiverer brain, and more rapidly than their fetal counterparts, achieving widespread and dense myelin basic protein expression by 4 weeks after graft. Adult OPCs generated oligodendrocytes more efficiently than fetal OPCs, and ensheathed more host axons per donor cell than fetal cells. Both phenotypes mediated the extensive and robust myelination of congenitally dysmyelinated host brain.

A broad range of pediatric leukodystrophies and storage diseases manifest with myelin failure or loss. Recent studies have focused on the use of transplanted oligodendrocytes or their progenitors for the treatment of congenital myelin diseases. The myelinogenic potential of implanted brain cells was first noted in the shiverer mouse [1,2]. Shiverer is an autosomal recessive mutation, and shi/shi homozygotes fail to develop myelin basic protein (MBP) or compact myelin, and die by 20-22 weeks. Transplanted fetal brain cells[3-6], both primary[7] and immortalized [8] neural progenitors, and enriched glial progenitor cells[9] have each been shown to myelinate shiverer axons, though all at low efficiency, as most of the implanted cells became astrocytes. Duncan similarly noted that rodent subventricular zone progenitors could engraft another dysmyelinated mutant, the myelin-deficient rat, upon perinatal intraventricular administration [10,11]. These studies suggested the attraction of using primary, isolated human oligodendrocyte progenitor cells (OPCs) as a means of myelinating congenitally dysmyelinated brain.

In this study, we investigated whether highly enriched populations of OPCs directly isolated from the human brain might be used as a substrate for cell-based therapy of congenital dysmyelination. Specifically, we postulated that human OPCs, whether derived from the fetal brain during its period of maximum oligoneogenesis, or from the adult subcortical white matter[12,13], could mediate the large-scale myelination of a congenitally dysmyelinated host. We

report here that both fetal and adult human OPCs, highly enriched via surface antigen-based fluorescence-activated cell sorting (FACS), were capable of widespread and high-efficiency myelination of the shiverer mouse brain after perinatal xenograft. In addition, we report significant differences in the behavior of fetal and adult-derived OPCs, which may recommend the therapeutic utility of one or the other for specific disease targets.

RESULTS

Cells dissociated from the late second trimester human ventricular zone of 21-23 weeks gestation were first magnetically sorted to isolate A2B5$^+$ cells[13-16]. These included both oligodendrocytic and neuronal progenitor cells. Since PSA-NCAM is expressed by immature neurons at this stage of development[17], we then used FACS to select out PSA-NCAM$^+$ neurons from the larger A2B5$^+$ cell population. This yielded a subpopulation of A2B5$^+$/PSA-NCAM$^-$ cells, which defined our oligodendrocyte progenitor pool. Two-color fluorescence-activated cell sorting (FACS) revealed that the A2B5$^+$/PSA-NCAM$^-$ fraction constituted 15.4 ± 4.8% of the cells in 21-23 week ventricular zone samples (n=5) (**Supplementary figure 1**). Among these PSA-NCAM-depleted A2B5$^+$ cells, 76.1 ± 0.5% expressed oligodendrocytic O4 by a week after FACS, whereas just 7.5 ± 0.3% expressed astrocytic glial fibrillary acidic protein (GFAP), and only 2.0 ± 1.3% neuronal βIII-tubulin. These data supported the glial restriction and oligodendrocytic bias of sorted A2B5$^+$/PSA-NCAM$^-$ cells. Since magnetic separation of A2B5$^+$ cells followed by FACS depletion of NCAM$^+$ cells achieved higher net yields than two-color FACS, we used this means of progenitor isolation.

Homozygote shi/shi mice were injected intracallosally with fetal progenitor cell isolates on either their day of birth or postnatal day 1. The animals were sacrificed thereafter at either 4, 8, 12 or 16 weeks of age. None of the animals were immunosuppressed; we relied on perinatal tolerization to ensure graft acceptance[18,19]. These injections yielded significant engraftment (defined as ≥100 cells per coronal section at 3 rostrocaudal levels, sampled >100 μm apart), in 34 of the 44 neonatal mice injected for this study (25 of 33 injected with fetal hOPCs, and 9 of 11 injected with adult-derived OPCs). By 12 weeks of age, the recipients exhibited donor engraftment throughout the callosum, capsular and commissural white matter, extending to the basis pontis caudally (**fig. 1A-E**). Over this time span, cell division among the engrafted

progenitors, though initially high at 4 weeks, fell to relatively low and stable levels at 8 and 12 weeks (**figs. 1F-G**).

During this same period, many of the fetal progenitors matured to become myelinogenic oligodendrocytes, as revealed by their expression of myelin basic protein (MBP). At 4 weeks, no detectable MBP was noted in 10 of 11 animals, despite widespread cell dispersion; scattered MBP^+ cells were noted in one mouse. At 8 weeks though, patchy foci of MBP expression were noted in 4 of 7 mice, and by 12 weeks, widespread MBP expression was noted throughout the forebrain white matter tracts in 5 of 7 mice. By this time, the engrafted mice typically expressed MBP throughout the entire extent of the corpus callosum, as well as throughout the fimbria and internal capsules (**figs. 2A-D**). Since shiverer mice express only the first exon of the MBP gene[2], and hence no immunodetectable MBP, any MBP detected in these recipients was necessarily donor-derived[8]. In addition, optical sectioning confirmed that the MBP^+ cells were of human origin, in that each MBP^+ profile was associated with an hNA^+ human soma (**fig. 2C, E-H**).

We next asked whether donor-derived myelin effectively wrapped host axons. To this end, we used confocal imaging and electron microscopy to assess axonal ensheathment and myelin compaction, respectively. Confocal analysis was first done on 3 shiverer brains that were each implanted on P1 with 100,000 fetal human OPCs, and sacrificed at 12 weeks. Foci of dense MBP expression were assessed by confocal imaging after immunolabeling for both human nuclear antigen and neurofilament protein, so as to tag donor-derived cells and host shiverer axons, respectively. By this means, human progenitors were found to have generated myelinating oligodendrocytes in great numbers. Among the recipients scored, 11.9 ± 1.6% (mean ± SE) of NF^+ host callosal axons were found to be surrounded by MBP-immunoreactivity (n=3 mice, with 3 fields scored/animal) (**figs. 3A-C**). We next used EM to verify that host axons were fully ensheathed by donor-derived oligodendrocytes, and that the latter generated compact myelin. Since MBP is required for compacting consecutive layers of myelin together, its expression is required for the major dense line of mature myelin. In the MBP-deficient shiverer, myelin failed to exhibit more than a few loose wrappings, and lacked major dense lines (**fig. 3D**). In contrast, the shi/shi graft recipients exhibited compact myelin with major dense lines (**figs. 3E-H**). Indeed, among a sample of MBP^+ fields (n=50), derived from 2 mice sacrificed 16 weeks after perinatal implant, an average of 7.4% of callosal axons (136 of 1832 sampled) had donor-derived myelin

sheaths, as defined by their major dense lines. Thus, engrafted fetal human OPCs efficiently differentiated as myelinogenic oligodendrocytes.

Some transplanted fetal OPCs differentiated as GFAP$^+$ astrocytes, as early as 4 weeks after implantation. Within white matter regions sampled on the basis of high donor cell engraftment, 12.7 ± 4.3% of fetal donor-derived cells expressed astrocytic GFAP at 12 weeks, while 10.2 ± 4.4% of donor cells expressed MBP. Importantly, no heterotopic βIII-tubulin or MAP2-defined neurons of donor derivation were noted at either 4, 8 or 12 weeks after implant (n = 33 total). Nonetheless, 40.3 ± 4.2% of donor cells expressed S100β, which is expressed by astrocytes and young oligodendrocytes, and nestin was expressed by 47.3 ± 4.2%, suggesting that a large proportion of donor cells persisted as glial progenitors after engraftment. Moreover, fetal OPCs were recruited as oligodendrocytes or astrocytes in a context-dependent manner, giving rise to both oligodendrocytes and fibrous astrocytes in the presumptive white matter, but only to GFAP$^+$ astrocytes in the gray matter (**fig. 2H and Supplementary figure 2**).

We next asked if adult-derived OPCs differed from their fetal counterparts with respect to their dispersal, myelinogenic capacity, or time courses thereof. We implanted 2 litters of P0 shiverer mice with A2B5-sorted OPCs extracted from adult human subcortical white matter. After 4, 8 or 12 weeks, the mice were killed and their brains stained for anti-human nuclear antigen and either MBP or GFAP; 9 of 11 exhibited successful engraftment. The adult OPCs achieved widespread and dense MBP expression by 4 weeks (**figs. 4A-B, 4E**); when assessed at 12 weeks, 39.5 ± 16.3% of adult OPCs expressed MBP. In contrast, no hNA$^+$ fetal donor OPCs expressed MBP at 4 weeks after engraftment, and 10.2 ± 4.4% did so by 12 weeks ($p < 0.001$ by 2-tailed t-test comparing the proportion of MBP$^+$ cells in fetal and adult-derived grafts) (**figs. 4A-C**). Thus, engrafted adult OPCs were at least four-times more likely to become oligodendrocytes and develop myelin than their fetal counterparts. Moreover, essentially no adult OPCs became astrocytes in the recipient white matter: none developed GFAP expression, whereas 12.7 ± 4.3% of fetal OPCs did so by 4 weeks. Thus, whereas nominally oligodendrocytic progenitors derived form the fetal brain actually acted as glial progenitors, adult OPCs behaved in a more restricted manner, largely generating either myelinogenic oligodendrocytes or persistent progenitors in recipient white matter. The greater rapidity of myelination by adult OPCs was reflected ultrastructurally, in that by 6 weeks after implant, the major dense lines of compact myelin were readily evident in mice implanted with adult OPCs at birth (**fig. 4D**). As noted, no such evidence

of myelin compaction was noted in mice implanted with fetal OPCs until 12-16 weeks postnatally.

Despite the apparent competitive advantage of adult OPCs, substantially higher numbers of fetal than adult donor cells engrafted in the recipient brains (**fig. 4F**). At the midline of the corpus callosum, the region of maximal engraftment, we scored 1123 ± 205.6 hNA^+ fetal donor cells/mm^2. Among these, 117 ± 43.7 were MBP^+, so that 9.8 ± 3.1% of fetal donor cells differentiated as myelinating oligodendrocytes by 12 weeks. In contrast, only 244 ± 182.1 donor cells/mm^2 were noted in the callosal midline of shiverer mice implanted with adult OPCs. Yet 81 ± 59.7, or 38.9 ± 12.9%, had developed as MBP^+ oligodendrocytes by 12 weeks ($p < 0.001$ by 2-tailed t-test comparing the proportion of MBP^+ cells in fetal vs. adult grafts) (**fig. 4G**). Moreover, whereas 12.7 ± 4.3% of fetal donor cells matured to express GFAP, no adult donor cells gave rise to $GFAP^+$ astrocytes, again suggesting a stronger bias towards oligodendrocytic phenotype by the adult progenitors. Thus, besides maturing more quickly than fetal OPCs, adult OPCs gave rise to oligodendrocytes in much higher proportions than their fetal counterparts .

To next assess whether adult and fetal OPCs differed in their individual extents of axonal ensheathment, we scored the numbers of axons myelinated by each donor OPC, as defined by confocal-verified MBP^+ enwrapment of NF^+ axons. These absolute values were then expressed as ratios to both total donor cell number and donor-derived MBP^+ oligodendrocytes per field. We found that when assessed 12 weeks after perinatal graft, adult-derived OPCs ensheathed many more host axons per donor cell than their fetal counterparts, and that this effect persisted even after limiting our analysis to the number of ensheathed axons per MBP^+ donor cell (**fig. 4H**). In each case, the difference between fetal and adult donor ensheathment efficiency was significant by Mann-Whitney analysis ($p < 0.02$). Thus, adult-derived OPCs matured to ensheath more axons per donor cell than their fetal counterparts.

DISCUSSION

These results indicate that enriched isolates of human OPCs, sorted from both the highly oligoneogenic late second trimester forebrain, as well as from the adult subcortical white matter, can broadly myelinate the shiverer mouse brain, a genetic model of perinatal leukodystrophy. When introduced as highly-enriched isolates, both fetal and adult-derived OPCs infiltrated widely throughout the presumptive white matter, ensheathed resident murine axons, and formed

antigenically and ultrastructurally compact myelin. Donor-derived myelinogenesis was geographically extensive, extending throughout all white matter regions of the telencephalon. After implantation, the cells slowed their mitotic expansion with time (see *supplemental data*), and generated neither undesired phenotypes nor parenchymal aggregates. Both fetal and adult-derived OPCs were competent to remyelinate murine axons, and neither generated heterotopic neurons. Nonetheless, sharp and important differences were noted: Whereas fetal OPCs were highly migratory, they myelinated slowly and inefficiently, and in the recipient white matter co-generated astrocytes as readily as myelinogenic oligodendrocytes. In contrast, adult-derived OPCs, extracted from the subcortical white matter using A2B5-based separation alone [13,20], migrated over lesser distances, but myelinated more rapidly and in higher proportions than did their fetal counterparts, with virtually no astrocytic co-production. Furthermore, on an individual basis, each adult OPC-derived oligodendrocyte ensheathed and myelinated substantially more axons than did its fetal-derived counterparts.

Together, these observations suggest that isolates of human glial progenitor cells may provide effective cellular substrates for remyelinating the congenitally dys- or hypomyelinated brain. In practical terms, the choice of stage-defined cell type may be dictated by both the availability of donor material, and by the specific biology of the disease target, since both fetal and adult OPCs proved competent to effect structural remyelination. That being said, both fetal and adult-derived human OPC isolates seem competent to achieve the widespread and efficient myelination of the congenitally dysmyelinated brain.

METHODS

Cells Fetal OPCs were extracted from human fetuses ranging from 21-23 weeks, obtained at abortion. The forebrain ventricular/subventricular zones were dissected free and chilled on ice. The minced samples were dissociated using papain/DNAse as described[21], always within 3 hrs of extraction, then maintained overnight in DMEM/F12/N1 with 20 ng/ml FGF. Adult-derived OPCs were harvested from subcortical white matter samples obtained at surgery, as described [12,13]. The 8 adult tissue samples used were largely derived from patients with medication-refractory epilepsy, undergoing temporal lobe resection. No tissues were accepted from patients with known neoplastic disease. Both fetal and adult samples were obtained with consent, under

approved protocols of the Cornell/New York Presbyterian Hospital and Albert Einstein College of Medicine/Jacoby Hospital Institutional Review Boards.

Sorting For fetal samples, on the day after dissociation the cells were incubated 1:1 with MAb A2B5 supernatant (clone 105; ATCC, Manassas, VA), for 30 min, then washed and labeled with either fluorophore or microbead-tagged rat anti-mouse IgM (Miltenyi Biotech). In some instances, 2-channel FACS was used to define the proportions and homogeneity of A2B5 and PSA-NCAM-defined subpopulations, using a FACSVantage SE/Turbo, as described[13,21]. For all preparative sorts for transplantation, A2B5$^+$ cells were prepared by magnetic separation (MACS; Miltenyi), following the manufacturer's protocol. The bound cells were eluted and incubated with mouse anti-NCAM (Pharmingen) at 1:25 for 30 min, then phycoerythrin-tagged anti-mouse at 1:200. The PSA-NCAM$^+$ population was then removed by FACS, leaving a highly enriched pool of A2B5$^+$/PSA-NCAM$^-$ cells. This PSA-NCAM immunodepletion step was omitted for adult samples, which were sorted on the basis of A2B5 only [12,20]. After sorting, both fetal and adult cells were maintained for 1-7 days in DMEM/F12/N1/bFGF (20 ng/ml), until implantation.

Transplantation and tagging Homozygous shiverers were bred in our colony. Within a day of birth, the pups were cryoanesthetized for cell delivery. 1 x 10^5 donor cells in 2 μl of HBSS were injected through a pulled glass pipette, inserted through the skull into the presumptive corpus callosum. Transplants were directed to the corpus callosum at a depth of 1.0 to 1.2 mm, depending on the weight of the pup, which varied from 1.0-1.5 g. The pups were killed at 4, 8, 12 or 16 weeks thereafter. For some experiments, recipient mice were injected for 2 days before sacrifice with BrdU (100 μg/g, as a 1.5 mg/100 μl solution), q12 hr for 2 consecutive days.

Immunohistochemistry The transplanted cells were identified using anti-human nuclei antibody (Chemicon; MAb 1281), CNP using Sternberger MAb 91 (Sternberger and Meyer, Inc.), S100 protein by a rabbit anti-S100 (Sigma), nestin by rabbit-anti human nestin (a gift of Dr, H. Okano), neurofilament by Sternberger MAb 311, human GFAP by Sternberger MAb 21, BrdU with an anti-rat BrdU (Harlan), and MBP by either Sternberger MAb 94 or Abcam 7349 (rat), all as described [7,12,20-22].

Confocal and electron microscopy Confocal imaging was performed using an Olympus Fluoview mated to an IX70 inverted microscope, as described[23]; argon laser lines were used to achieve 3-channel immunofluorescent detection of fluorescein, Texas Red and Cy5 tagged antibodies; the latter was then pseudocolored blue for presentation. For EM, animals were

perfused and postfixed with 4% paraformaldehyde and 0.25% glutaraldehyde in 6% sucrose phosphate buffer (sucrose-PB), then Vibratome sectioned as alternating thick (400 μm) and thin (100 μm) sections. The latter were immunostained for MBP; thick sections adjacent to thins exhibiting MBP expression were then processed in 1% osmium-1.5% ferricyanide, stained with 1.5% uranyl acetate, then embedded in Epon, cut as 100 nm thin sections onto Formvar-coated grids, stained with lead citrate and visualized using a Jeol 100 electron microscope[24].

Supplemental figure 1

Fluorescence-activated sorting of fetal human oligodendrocyte progenitor cells

This plot shows the result of dual-color FACS of a 23 week human fetal ventricular zone dissociate, after concurrent immunostaining for both A2B5 (*green*) and PSA-NCAM (*red*). The FACS plot on the left (**A**) illustrates a matched but unstained 23 week dissociate. On the right, **B** shows the same VZ dissociate, sorted after dual immunolabeling for A2B5 (FL1, *y* axis) and PSA-NCAM (FL2, *x* axis). The A2B5$^+$/PSA-NCAM$^-$ fraction in R1R3, comprising 16.5% of the dissociate, corresponded to glial progenitor cells. Although these were able to generate both astrocytes and oligodendrocytes, they were preferentially oligoneogenic when derived at this gestational age, and were thus designated as oligodendrocyte progenitor cells (OPCs). In contrast, the R1R5 fraction, defined by the antigenic phenotype A2B5$^-$/PSA-NCAM$^+$, generated largely neurons in vitro (*not shown*), and was therefore defined as a neuronal progenitor pool.

C-D shows, at lower and higher magnification respectively, the presence of O4$^+$ oligodendrocytes generated from the A2B5+/PSA-NCAM- fraction four days post-FACS. In contrast, the R1R5 fraction, defined by the antigenic phenotype A2B5-/PSA-NCAM+, generated largely neurons in vitro (*not shown*), and was therefore defined as a neuronal progenitor pool.

E and **F** plot the phenotypic composition of sorted fetal oligodendrocytic and neuronal progenitor cells, respectively defined by the antigenic phenotypes A2B5$^+$/PSA-NCAM$^-$ (**E**) and A2B5$^-$/PSA-NCAM$^+$ (**F**), and sorted within the R1R3 and R1R5 fractions of 1B. Each isolate was immunostained for oligodendrocytic O4, neuronal βIII-tubulin, astrocytic GFAP, and nestin as a marker of uncommitted and immature phenotypes. Scale: **C**, 60 μm; **D**, 30 μm.

Supplemental figure 2

OPCs were recruited as oligodendrocytes or astrocytes in a context-dependent manner

In the presumptive white matter, implanted OPCs typically matured as either MBP^+ myelinogenic oligodendrocytes in the presumptive white matter, or less so as $GFAP^+$ astrocytes. In contrast, in the recipient cortex and subcortical gray matter, donor cells gave rise solely to GFAP-defined astrocytes. **A**, as in **fig. 2F**, shows the striatocallosal border of a shiverer brain, 3 months after perinatal engraftment with human fetal OPCs (hNA in *blue*). Donor-derived MBP (*red*) expression is evident in the corpus callosum, while donor-derived $GFAP^+$ (*green*) astrocytes predominate on the striatal side. Here, in **B-D** the individual color splits reveal the sharp borders between white matter-associated MBP^+ oligodendrocytes and striatal $GFAP^+$ astrocytes, both derived from a common injection of nominally homogeneous fetal OPCs.

Scale = 200 μm.

Figure 1

Fetal human OPCs disperse rapidly to infiltrate the forebrain

Human donor cells dispersed widely by 12 weeks after perinatal implantation into shiverer recipients. The human cells were localized by anti-human nuclear antigen (hNA) immuno-staining; low-power fluorescence images were collected at representative anteroposterior levels (**shown in A**) and schematized[25]. In **B-E**, the engrafted cells (in *red*) are seen to have dispersed widely throughout the forebrain, although most remain in the subcortical white matter tracts.

F shows the mitotic activity of engrafted progenitors at 4 and 12 weeks after xenograft. The shiverer recipients were given intraventricular injections of sorted human OPCs on postnatal day 1, then injected with BrdU (100 μg/g, i.p.) twice daily for 2 days prior to sacrifice. Mitotic human OPCs were observed as $BrdU^+/hNA^+$ cells (*arrows*).

G, a regression of the incidence of mitotically-active donor cells as a function of time after perinatal implant. The fraction of human donor cells that incorporated BrdU during the 48 hrs preceding sacrifice dropped from 42 ± 6.1% at 4 weeks, to 8.2 ± 2.4% at 12 weeks. Statistical analysis revealed that the rate of BrdU incorporation declined according to the exponential regression: $y = 83.4e^{-0.22x}$, with a correlation coefficient of $r = -0.87$ ($p = 0.012$).

Scale: **B-E**, 3 mm; **F**, 50 μm

Figure 2

Engrafted human OPCs myelinate an extensive region of the forebrain

A-B, Extensive myelin basic protein expression by sorted human fetal OPCs, implanted into homozygote shiverer mice as neonates, indicates that large regions of the corpus callosum (**A** and **B**, 2 different mice) have myelinated by 12 weeks (MBP, *green*). **C,** human OPCs also migrated to and myelinated fibers throughout the dorsoventral extents of the internal capsules, manifesting widespread remyelination of the forebrain after a single perinatal injection.

D, Myelin basic protein expression (*green*), in an engrafted shiverer callosum 3 months after perinatal xenograft, is associated with human donor cells, identified by human nuclear antigen (hNA, in *red*). Both the engrafted human cells and their associated myelin were invariably found to lay parallel to callosal axonal tracts.

E, Confocal optical sections of implanted shiverer corpus callosum, with human cells (hNA, in *red*) surrounded by myelin basic protein (MBP, *green*). Human cells (*arrows*) found within meshwork of MBP^+ fibers (merged image to left, with 3 images to right taken 1 μm apart).

F, OPCs were recruited as oligodendrocytes or astrocytes in a context-dependent manner. This photo shows the striatocallosal border of a shiverer brain, 3 months after perinatal engraftment with human fetal OPCs (hNA in *blue*). Donor-derived MBP^+ oligodendrocytes and myelin (*red*) is evident in the corpus callosum, while donor-derived $GFAP^+$ (*green*) astrocytes predominate on the striatal side.

Scale: **A-C**, 1 mm; **D**, 100 μm; **E**, 20 μm; **F**, 200 μm.

Figure 3

Axonal ensheathment and myelin compaction by engrafted human progenitor cells

A, a confocal micrograph showing a triple-immunostain for MBP (*red*), human ANA (*blue*), and neurofilament protein (*green*). In this image, all MBP immunostaining is derived from the sorted human OPCs, whereas the NF^+ axons are those of the mouse host. *Arrows* identify segments of murine axons ensheathed by human oligodendrocytic MBP.

B, a 2 μm deep composite of optical sections, taken through the corpus callosum of a shiverer recipient sacrificed 12 weeks after fetal OPC implantation. Shiverer axons were scored as ensheathed when the *yellow* index lines intersected an NF^+ axon abutted on each side by MBP-immunoreactivity. The *asterisk* indicates the field enlarged in **C**.

C, At higher magnification, MBP-immunoreactivity (*red*) can be seen to surround ensheathed axons (*green*) on both sides.

D, Electron micrographs of a sagittal section through the corpus callosum of an adult shi/shi homozygote. Shiverer axons typically have a single loose wrapping of myelin that fails to compact, so that major dense lines fail to form.

E-H, Representative electron micrographs of 16-week old shiverer homozygotes, implanted with human oligodendrocyte progenitor cells shortly after birth. These images show resident shiverer axons with densely compacted myelin sheaths. The *asterisk* indicates the field enlarged in the inset. **Inset**, Major dense lines are noted between myelin lamellae, providing EM confirmation of myelination by engrafted human OPCs.

Scale bar = **A**, 20 μm; **B**, 40 μm; **C-F**, 1 μm.

Figure 4

Fetal and adult OPCs differed substantially in their speed and efficiency of myelinogenesis

A, Adult-derived human OPCs (hNA, *red*) achieved dense MBP expression (*green*) by 4 weeks after xenograft. **B,** In contrast, fetal OPCs expressed no detectable MBP-IR at 4 weeks, with such expression not noted until 12 wks. **C,** Low and high (*inset*) power coronal images of the callosal-fimbrial junction of a shiverer homozygote, showing dense myelination 12 weeks after perinatal engraftment with adult human OPCs. **D,** Adult OPCs developed mature myelin ultrastructure and major dense lines within 5 weeks of perinatal injection. **These** examples show myelin in a shiverer homozygote 5 weeks after perinatal injection of adult OPCs. The myelin structure seen here was analogous to that of fetal OPCs assessed 12 weeks after implant (see **fig. 3**). Mice injected with fetal OPCs exhibited no such evidence of myelination at this early timepoint.

E, the distribution of hNA^+ adult OPCs (*red*), 4 weeks after implantation into shiverer homozygotes (compare to **fig. 2**). **F,** A higher proportion of adult OPCs developed MBP expression than did fetal OPCs, when both were assessed 12 weeks after transplant. **G,** Fetal OPCs nonetheless engrafted more efficiently and in higher numbers than did adult OPCs. * $p<0.05$; ** $p<0.005$, Student's t-test (2-tailed).

H, This graph plots the numbers of MBP-ensheathed NF^+ axons per donor cell, as achieved by fetal and adult-derived OPCs. Ensheathment was measured as a function both of total donor cell number (*left* panel), and of MBP^+ donor-derived oligodendrocytes (*right*). The differences between fetal and adult donor ensheathment efficiencies were significant by Mann-Whitney analysis ($p < 0.02$).

Scale: **A-B**, 100 μm, **C**, 1 mm; inset, 30 μm; **D**, 1 μm.

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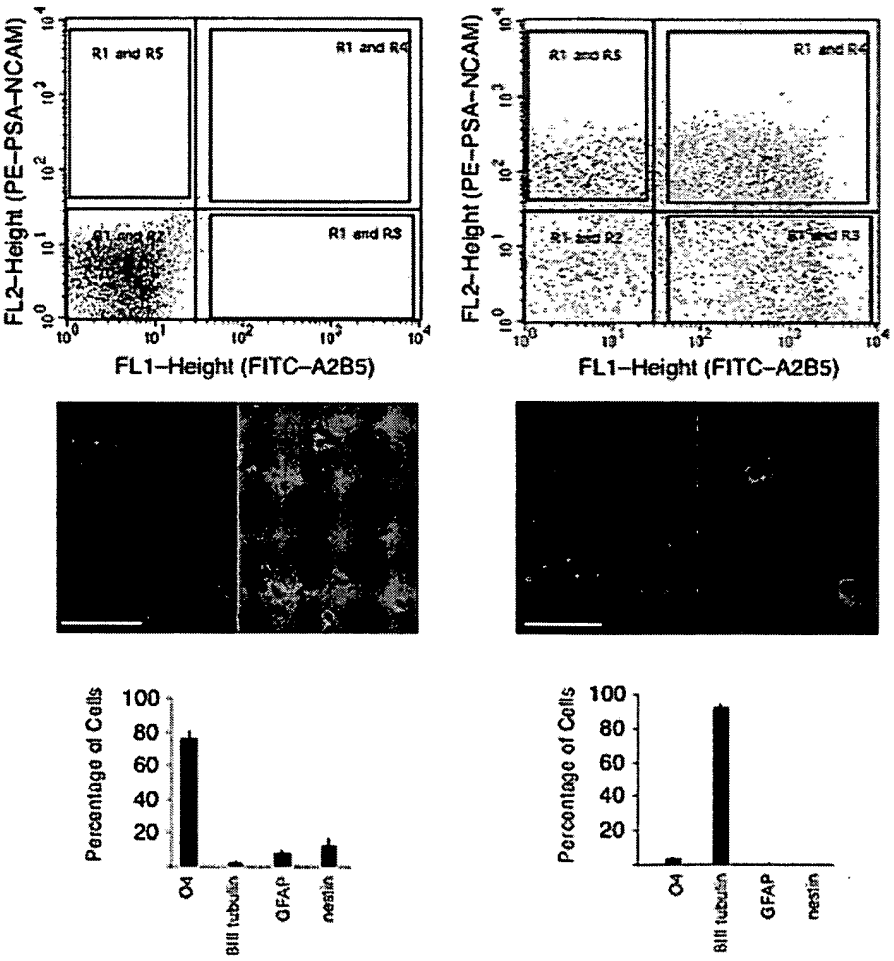
supplemental figure 1



Supplementary figure 2

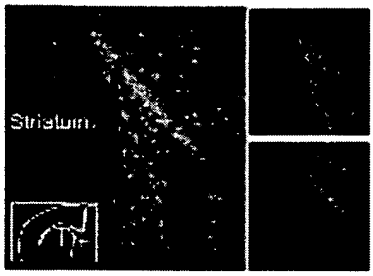


Figure 1

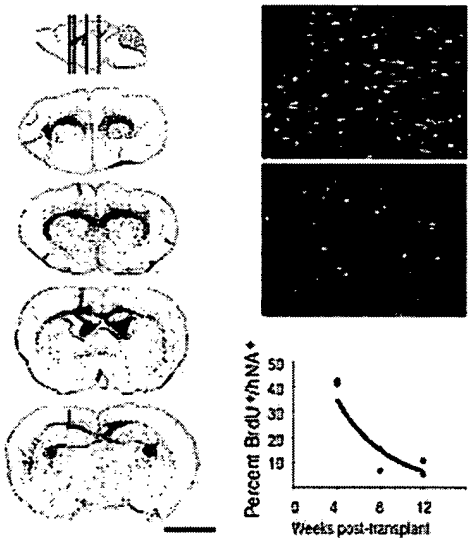
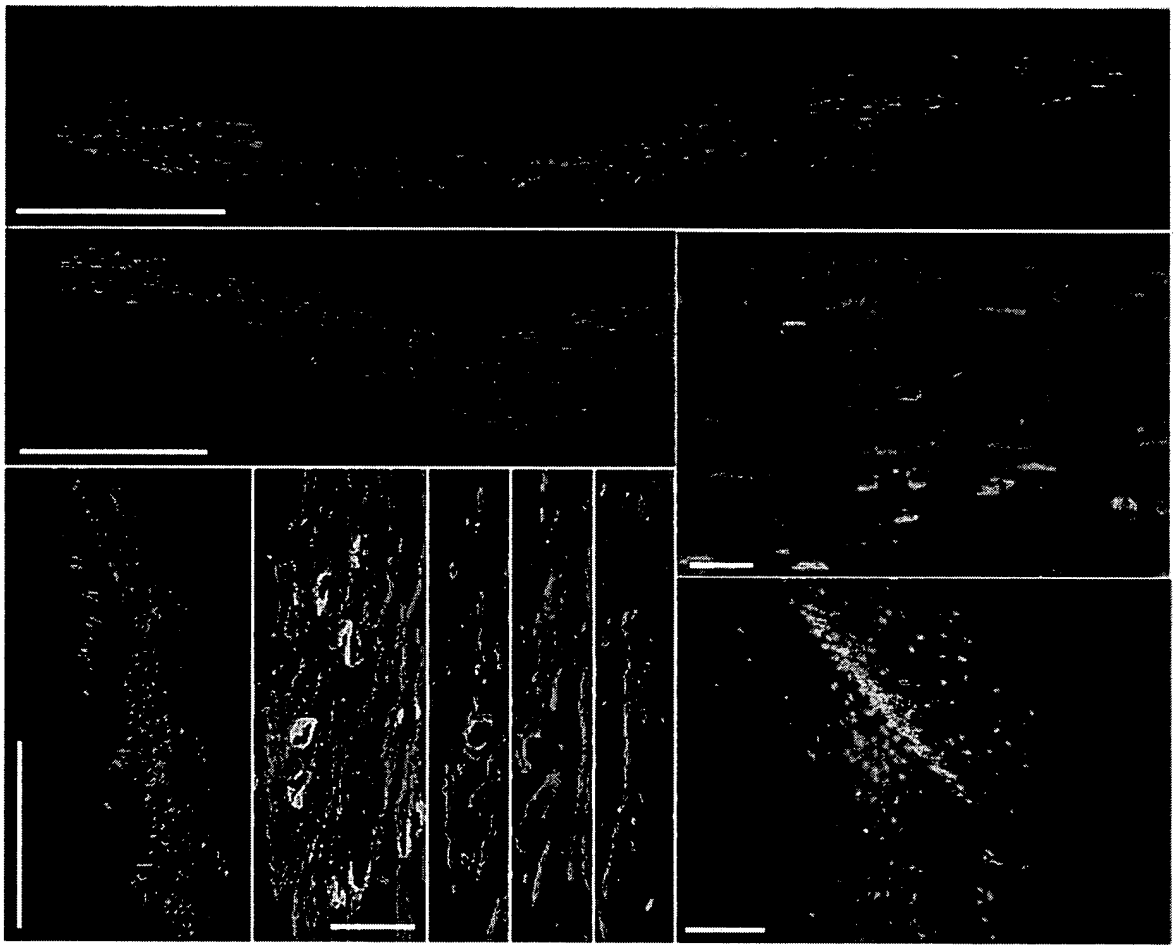


Figure 2



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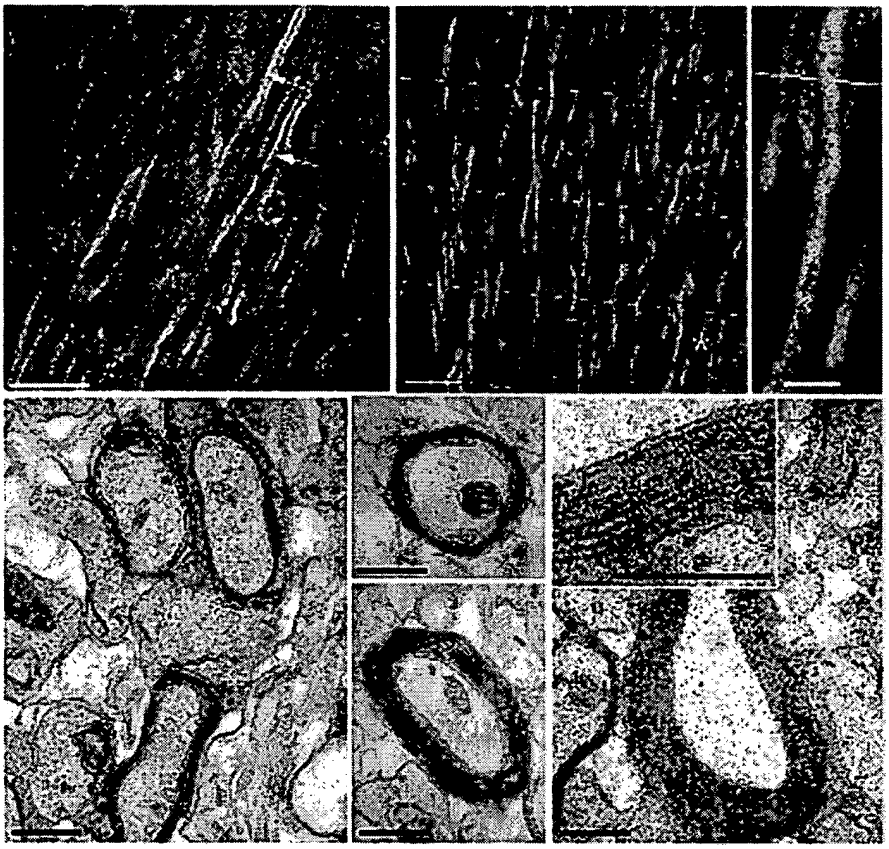

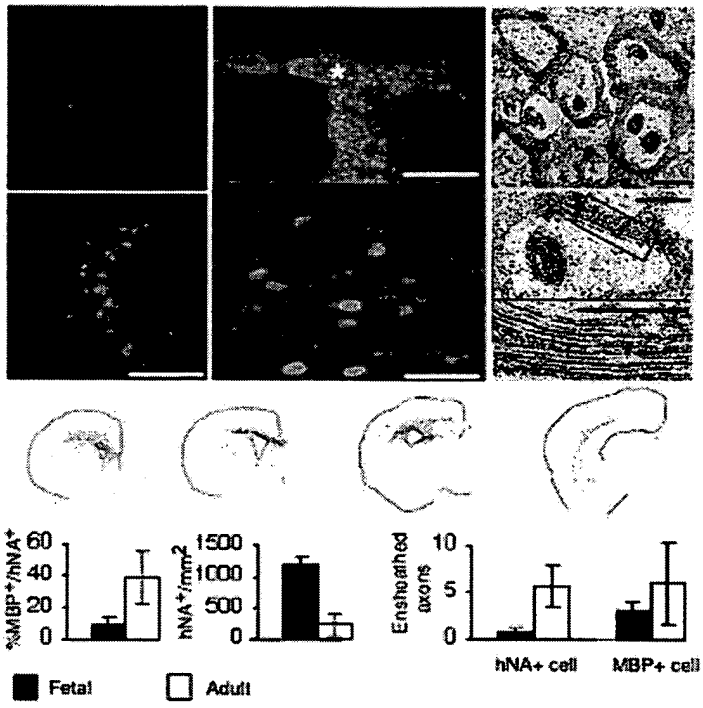
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